

AMENDMENTS TO THE CLAIMS:

This listing of claims will replace all prior versions, and listings, of claims in the application:

LISTING OF CLAIMS:

Claim 1. (Currently Amended) A method for the determination of the absolute concentration ( $\text{sperm}/\text{ml}$ ) of sperm cells in a semen sample and the proportion of live sperm cells therein, comprising subjecting the semen sample to selective staining, wherein said selective staining comprises applying a first fluorochrome which binds to DNA and stains all sperm cells and a second fluorochrome which binds to DNA and selectively stains dead and dying cells, and determining the absolute concentration of the sperm cells and the proportion of live sperm cells by means of a detection means responsive to the selective staining, wherein the determination of the absolute concentration of sperm cells and of the proportion of live sperm cells in the semen sample are performed simultaneously in the same determination step.

Claim 2-4 (Cancelled).

Claim 5. (Previously Presented) A method according to claim 1, wherein any dilution of the sample has been performed using a diluent which sustains viability of the sperm cells during the determination.

Claim 6. (Previously Presented) A method according to claim 1, wherein the selective staining is performed using one or more fluorochromes resulting in fluorescent qualities being conferred to live sperm cells and dead sperm cells, the fluorescent quality or qualities of live sperm cells being distinguishable, by the detection means, from the fluorescent quality or qualities of dead sperm cells, and the determination is performed by selective counting of cells of each fluorescent quality.

Claim 7. (Previously Presented) A method according to claim 1, wherein the proportion of dying sperm cells is also determined, the selective staining being adapted to allow distinction,

by the detection means, between dying sperm cells and on the one hand dead sperm cells and on the other hand live sperm cells.

Claim 8. (Original) A method according to claim 7, wherein the selective staining is performed using one or more fluorochromes resulting in fluorescent qualities being conferred to live sperm cells, dead sperm cells and dying sperm cells, the fluorescent quality or qualities of live sperm cells, dead sperm cells and dying sperm cells being distinguishable from each other by the detection means, and the determination is performed by selective counting of cells of each fluorescent quality.

Claim 9. (Cancelled)

Claim 10. (Previously Presented) A method according to claim 1, wherein said second fluorochrome being capable of entering a sperm cell through a leaking or defect plasma membrane, but substantially incapable of entering a sperm cell having an intact plasma membrane, and said first fluorochrome being capable of entering a cell through an intact cell membrane.

Claim 11. (Previously Presented) A method according to claim 6, wherein the excitation of the fluorochromes is performed by means of light in the wavelength range about 488 nm, the first fluorochrome staining all sperm cells being SYBR-14, and the second fluorochrome staining the dead or dying sperm cells-being propidium iodide.

Claims 12-13 (Cancelled)

Claim 14. (Previously Presented) A method according to claim 11, wherein the first fluorochrome staining all sperm cells is used in total concentrations in the range from 25 to 75 nanomolar.

Claim 15. (Previously Presented) A method according to claim 14, wherein the first fluorochrome staining all sperm cells is used in total concentrations about 50 nanomolar.

Claim 16. (Previously Presented) A method according to claim 1, wherein the staining of the sperm cells is performed at a temperature below 35°C.

Claim 17. (Previously Presented) A method according to claim 16, wherein the staining of the sperm cells is performed at a temperature of at the most 30°C.

Claim 18. (Previously Presented) A method according to claim 17, wherein the staining of the sperm cells is performed at a temperature between 15°C and 25°C.

Claim 19. (Previously Presented) A method according to claim 18, wherein the staining of the cells is performed at room temperature.

Claim 20. (Previously Presented) A method according to claim 1, wherein the sample or a subsample is combined with an internal concentration standard means, and the determination of the absolute concentration of the sperm cells and the proportion of live sperm cells are performed simultaneously by means of a detection means responsive to the selective staining and to the internal concentration standard means.

Claim 21. (Previously Presented) A method according to claim 20, wherein the internal concentration standard means is constituted by standardization particles, the standardization particles being added in a predetermined number per weight or volume amount of the sample or subsample.

Claim 22. (Previously Presented) A method according to claim 20, wherein the standardization particles are fluorescent particles having a fluorescent quality distinguishable from the fluorescent qualities of the live sperm cells, dead sperm cells, and dying sperm cells.

Claim 23. (Previously Presented) A method according to claim 20, wherein the detection means comprises a flow cytometer.

Claim 24. (Previously Presented) A method according to claim 20, wherein the detection means comprises a laser scanning cytometer.

Claim 25. (Currently Amended) A method according to claim 21, wherein the size and total sperm cell concentration absolute concentration of sperm cells of the subsample are adapted so that the number of sperm cells corresponds to between one tenth and ten times the number of standardization particles.

Claim 26. (Currently Amended) A method according to claim 25, wherein the size and total sperm cell concentration absolute concentration of sperm cells of the subsample are adapted so that the number of sperm cells corresponds to between one quarter and four times the number of standardization particles.

Claim 27. (Currently Amended) A method according to claim 26, wherein the size and total sperm cell concentration absolute concentration of sperm cells of the subsample are adapted so that the number of sperm cells corresponds to between half and twice the number of standardization particles.

Claim 28. (Previously Presented) A method according to claim 5, wherein the diluent is a diluent containing protein.

Claim 29. (Previously Presented) A method according to claim 28, wherein the protein is BSA.

Claim 30. (cancelled)

Claim 31. (Previously Presented) A method according to claim 1, wherein the determination of the absolute concentration of the sperm cells and the proportion of live sperm cells are determined as a mean value of the determination of the absolute concentration of the sperm cells and the proportion of live sperm cells performed on two or more subsamples of a semen sample.

Claim 32. (Previously Presented) A method for predicting the likelihood of fertilizing a female animal by artificial insemination with an insemination dose, comprising determining the absolute concentration of sperm cells in the semen sample from which the insemination dose is taken or is to be taken, and the proportion of live sperm cells therein by a method according to claim 1, and including the thus determined absolute concentration of the sperm cells in the semen sample and the proportion of live sperm cells therein, or the concentration, calculable therefrom, of live sperm cells in the sample, on the basis of which the likelihood of fertilizing the animal is predicted.

Claim 33. (Previously Presented) A method according to claim 32, wherein the likelihood of fertilizing the female animal is predicted on the basis of the determined absolute concentration of the sperm cells in the semen sample and the proportion of live sperm cells therein, or the concentration, calculable therefrom, of live sperm cells in the sample.

Claim 34. (Currently Amended) A method according to claim 32, wherein the prediction of the likelihood of fertilizing the female animal is performed on the basis of statistically significant correlations between fertility data obtained in insemination experiments with several female animals and data indicating one or more of the following: the absolute concentration of the sperm cells in the semen sample used in the insemination experiments, and the proportion of live sperm cells therein, and/or data indicating the concentration of live sperm cells therein.

Claim 35. (Previously Presented) A method according to claim 32, wherein the female animal is a multiparous animal, and the number of offspring resulting from the fertilization is also predicted.

Claim 36. (Previously Presented) A method according to claim 32, wherein the semen sample is a fresh ejaculate.

Claim 37. (Previously Presented) A method according to claim 32, wherein the semen sample is a frozen insemination dose, the sample being thawed before being subjected to the determination method.

Claim 38. (Previously Presented) A method according to claim 37, wherein data obtained by the determination method performed on the fresh ejaculate from which the insemination dose was taken are included together with data obtained by the determination method performed on the insemination dose.

Claim 39. (Previously Presented) A method for artificial insemination of a female animal, comprising predicting the likelihood of fertilizing a female animal, the prediction comprising determining the absolute concentration of sperm cells in the semen sample from which the insemination dose is taken or is to be taken, and the proportion of live sperm cells therein by a method according to claim 1, and including the thus determined absolute concentration of the sperm cells in the semen sample and the proportion of live sperm cells therein, or the concentration, calculable therefrom, of live sperm cells in the sample on the basis of which the likelihood of fertilizing the animal is predicted, and on basis of the predicted likelihood selecting an insemination dose for use with artificial insemination of the female animal.

Claim 40. (Previously Presented) A method according to claim 39, wherein the female animal is a multiparous animal, and the insemination dose is an insemination dose having a predicted likelihood of resulting in a number of offspring above a predetermined discrimination number.

Claim 41. (Previously Presented) A method according to claim 39, wherein the likelihood of fertilizing the female animal is predicted on the basis of the determined absolute concentration

of the sperm cells in the semen sample and the proportion of live sperm cells therein, or the concentration, calculable therefrom, of live sperm cells in the sample.

Claim 42. (Currently Amended) A method according to claim 39, wherein the prediction of the likelihood of fertilizing the female animal is performed on the basis of statistically significant correlations between fertility data obtained in insemination experiments with several female animals and data indicating one or more of the following: the absolute concentration of the sperm cells in the semen sample used in the insemination experiments, and the proportion of live sperm cells therein, and/or data indicating the concentration of live sperm cells therein.

Claim 43. (Previously Presented) A method according to claim 39, wherein the semen sample is a fresh ejaculate.

Claim 44. (Previously Presented) A method according to claim 39, wherein the semen sample is a frozen insemination dose, the sample being thawed before being subjected to the determination method.

Claim 45. (Previously Presented) A method according to claim 1 wherein the determination of the absolute concentration of sperm cells is performed in the same determination routine.

Claims 46-49. (Canceled).

Claim 50. (Previously Presented) A method according to claim 1, wherein the semen sample is a diluted subsample of said semen sample.